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(54) **Real-time scanning fluorescence electrophoresis apparatus for the analysis of polynucleotide fragments**

Vorrichtung zur Echtzeitanalyse von Polynucleotidfragmenten durch fluoreszierende Abtastelektrophorese

Appareil d'electrophorese par fluorescence a balayage en temps reel destine a l'analyse de fragments de polynucleotides

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Description

FIELD OF THE INVENTION

This invention relates to improved apparatus for performing electrophoresis, and more particularly to an improved real-time scanning fluorescence electrophoresis apparatus for polynucleotide fragment analysis.

BACKGROUND OF THE INVENTION

Electrophoretic polynucleotide fragment analysis methods are used to characterize mixtures of polynucleotide fragments based on their migration velocity through a polymer network under the influence of an electric field, i.e. their electrophoretic mobility, in combination with single or multi-color fluorescence detection. Typically these methods are applied subsequent to amplification of the target polynucleotide using a method such as PCR, e.g. Mullis, U.S. patent 4,683,202 Examples of such methods include polynucleotide sequencing, e.g. Trainor, Anal.Chem., 62: 418-426 (1990), restriction fragment length polymorphisim (RFLP) analysis, e.g. Watkins, Biotechniques, 6: 310-319 (1988), and variable number of tandem repeat (VNTR) or microsatellite analysis, e.g. Ziegle et al., Genomics, 14: 1026-1031. Each ofthese methods can provide valuable genetic information about the target polynucleotide.

Current electrophoretic polynucleotide fragment analysis systems are characterized by multiple electrophoresis lanes arranged in a planar array, e.g. a multilane slab gel, in combination with a real-time-scanning fluorescence detector, e.g. Hunkapiller et al., U.S. patent 4,811,218. Multiple lanes are used to increase the overall throughput of the analyzer. In order to collect data during the electrophoresis from multiple lanes, the optical detector system is scanned across the width of the electrophoresis chamber perpendicular to the direction of migration of the labeled polynucleotides. Preferably, multi-color fluorescence detection is used to increase the information density per lane, e.g. for DNA sequencing, four label colors are used, one color for each base. A light source, e.g. a laser, excites the fluorescent labels attached to the polynucleotide fragments, and multiple emission filters discriminate between labels having different spectral properties. In addition, a computer is used to collect data consisting of time, lane number, and fluorescence emission wavelength information, and transform it into useful information, e.g. DNA sequence.

A significant limitation on the speed and resolution of current polynucleotide fragment analysis systems is the ability to dissipate the Joule heat that is generated as a result of the electric current passing through the electrophoresis medium. Because of problems caused by Joule heating, current systems are limited to low, e. g. 25 V/cm, electrical fields, resulting in long analysis times, e.g. 8 hrs. Joule heating and the resulting temperature gradient across the gel can negatively impact the quality of the separation in two ways. First, because heat is generated throughout the electrophoresis medium but only dissipated at its' outside surfaces, a parabolic temperature profile is establish across the depth of the channel. Since electrophoretic velocity is a strong function of temperature, approximately 2% per °C, this temperature profile leads to a parabolic velocity profile for the migrating analytes. This spatial dependence of velocity causes a broadening of the migrating zones, leading to reduced separation performance. The extent of the temperature profile can be reduced by making the electrophoresis channel thinner, e.g. Brumley et al., Nucleic Acids Research, 19: 4121-4126 (1991); Stegemann et al., Methods in Molecular and Cellular Biology, 2: 182-184 (1991). Therefore, an automated system which incorporates thin electrophoresis channels would be desirable.

Second, if the average temperature of the electrophoresis medium becomes too high, the structural integrity of the medium can be compromised. In the case of polymer gel media, e.g. crosslinked polyacrylamide gels, the elevated temperature can lead to complete destruction of the gel. The average temperature of the electrophoresis medium can be controlled by increasing the rate of heat transfer from the electrophoresis channel to the surrounding environment. Therefore, a system which more efficiently transfers the Joule heat generated as a result of the electrophoresis to the surrounding environment would be desirable.

A further limitation on the speed and resolution of electrophoretic separations is the rate at which the detector can acquire data from fast moving analyte bands. The most desirable form of detection for polynucleotide fragment analysis would be simultaneous multi-color detection. However, current approaches, i.e. an indexable filter wheel in combination with a photomultiplier tube (PMT) detector, are not ideal because the filter wheel must be indexed rapidly enough to observe each color before it moves out of the detector region. This is problematic due to the high electrophoretic velocity of the polynucleotide fragments in high-speed systems. If a sufficient number of data points are not collected for each analyte band, e.g. 10 points per band, the ability to discriminate between adjacent bands is lost. One way to increase the rate of data acquisition for a multi-color system is to collect signals from all the colors simultaneously rather than serially. Therefore, a detection system which acquires all colors simultaneously would be desirable.

In light of the above, what was needed was an improved electrophoresis apparatus capable of accommodating high electric fields through enhanced heat dissipation characteristics and detector performance.

SUMMARY OF THE INVENTION

The present invention is directed to improvements

to an apparatus for electrophoretic polynucleotide analysis, said improvements leading to increased throughput of the system. The improvements include (i) incorporating a spectral-array detector to increase the rate of data acquisition, and (ii) incorporating an improved means to control the temperature of the electrophoresis medium. The analyzer system of the present invention is comprised of, in combination,

An improved real-time scanning fluorescence electrophoresis apparatus for the electrophoretic analysis of fluorescently-labeled polynucleotide fragments of the type having an electrophoresis chamber containing an electrophoretic separation medium capable of accommodating multiple electrophoresis lanes arranged in a planar array, a fluorescence detector mounted on a translatable stage, a light source for exciting fluorescent molecules, and a computer for collecting data consisting of time, location, fluorescence wavelength and fluorescent intensity information wherein the improvement comprises:

(a) a spectral-array detector for detecting the emission light from said fluorescently-labeled polynucleotide fragments including the simultaneous detection of multiple fluorescent labels,
(b) a temperature control means to control the temperature of the electrophoretic separation medium during electrophoresis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a vertically oriented slab gel.

Figure 2 shows a schematic diagram of the light path in a preferred embodiment of the spectral-array detection system of the present invention.

Figures 3 shows a plate holder according to a prefered embodiment of the invention.

Figure 4 shows a plate locating mechanism according to a prefered embodiment of the invention. .

Figure 5 shows a temperature control mechanism according to. a prefered embodiment of the invention.

DEFINITIONS

The term "polynucleotide" as used herein refers to linear polymers of natural or modified nucleoside monomers, including double and single stranded deoxyribonucleosides, ribonucleosides, α-anomeric forms thereof, and the like. Usually the nucleoside monomers are linked by phosphodiester bonds or analogs thereof to form polynucleotides ranging in size from a few monomeric units, e.g. 8-40, to several thousands of monomeric units. Whenever a polynucleotide is represented by a sequence of letters, such as "ATGCCTG," it will be understood that the nucleotides are in 5'-3' order from left to right and that "A" denotes deoxyadenosine, "C" denotes deoxycytidine, "G" denotes deoxyguanosine, and "T" denotes thymidine, unless otherwise noted. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like.

As used herein, "nucleoside" includes the natural nucleosides, including 2'-deoxy and 2'-hydroxyl forms, e.g. as described in Kornberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992). "Analogs" in reference to nucleosides includes synthetic nucleosides having modified base moieties and/or modified sugar moieties, e.g. described generally by Scheit, Nucleotide Analogs (John Wiley, New York, 1980).

As used herein, the term "electrophoretic separation medium" refers to a material through which the polynucleotides are electrophoresed and which imparts a size-dependent electrophoretic velocity to the polynucleotides. Typically, such material is a porous network formed by linear or branched polymer molecules, or the like, e.g. crosslinked polyacrylamide.

As used herein, the term "electrophoresis chamber" refers to the container in which the electrophorertic separation is contained. Typically, this container is formed by two rectangular glass plates which are separated by thin polymer sheets, spacers, located between the plates at the edge regions of the plates. This is traditionally referred to as slab electrophoresis. When the electrophoretic separation medium is a rigid crosslinked gel, this format is referred to as slab gel electrophoresis.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Figure 1 shows polynucleotide fragment samples (2) which have been labeled with one of several fluorophores loaded into loading wells (4) of vertically oriented slab gel (8), said gel mounted in the analyzer of the present invention. The fragments are electrophoresed through gel (8) where they are separated based on their relative size. Following separation, the fragments pass through laser excitation and detection region (12) where the fluorescently labeled polynucleotide fragments are detected. The fluorophores emit light at a specific wavelength based upon the particular dye used, thereby facilitating the identification of each fragment.

After the polynucleotide fragments have been separated, they are detected by a simultaneous multi-color detection means. An important feature ofthe polynucleotide analyzer of the present invention is the "spectral-array fluorescence detector". As used herein, the term "spectral- array fluorescence detector" refers to a detector which employs (i) a means to spectrally separate the fluorescence emission light, such as a diffraction grating, or a prism, or the like, (ii) an array of detector elements sensitive to light radiation, such as a diode array, a charged coupled device (CCD) system, an array of photomultiplier tubes, or the like, (iii) an excitation light source, such as an incandescent bulb, an arc lamp, a laser, a laser diode, or the like, and (iv) associated optics

capable of directing and conditioning both the excitation and emission light. The output of a spectral-array detector is light intensity as a function of array location, wherein the array location can be directly related to the wavelength of the light falling on that location. One example of such a detector is given by Karger et al., Nucleic Acids Research 19: 4955-4962 (1991).

One preferred method of treating the output of a spectral-array detector is to create a "virtual filter". As used herein, the term "virtual filter" refers to a method of manipulating data from a spectral-array detector such that a plurality of discrete wavelength ranges are sampled, wherein the location and bandwidth of each wavelength range can be dynamically changed using software. The virtual filter can mimic a physical interference or absorbence filter, however it has several important advantages. First, virtual-filters can be programmed to interrogate multiple emission wavelengths simultaneously, making possible the efficient multi-color detection of fast-moving analytes without the need to rapidly index a multiplicity of filters. Second, virtual filters can be programmed to detect a range of emission bandwidths. This is important because for any application, there exists an optimum bandwidth which results in an optimum combination of sensitivity and color discrimination: as the detection band width is made wider, the detector collects more light, thereby increasing sensitivity, however, at the same time, the broader bandwidth decreases the ability to discriminate between closely related colors. Third, virtual filters have essentially perfect transmission curves, i.e. the filter can discriminate between very closely related colors. Forth, the selected wavelength ranges of the virtual filter can be easily adjusted using software to match the characteristics of various excitation light sources and dye sets. Therefore, changing dye chemistries is a simple matter of changing the virtual filter with software, whereas a mechanical modification of the system is required when physical filters are used. Moreover, the selected wavelength ranges and band widths of the virtual filter can be changed dynamically, i.e. during the course of a run, to compensate for any spectral changes in the dye labels which occur during a run.

Figure 2 is a schematic diagram of the light path in a preferred embodiment of the spectral-array detection system of the present invention. Preferably, the analyzer system of the invention uses a laser as a fluorescence excitation light source, e.g. an argon ion laser that emits a 40 mW, 0.67 mm diameter polarized light beam having intensity maxima at wavelengths of 488 and 514 nm. Light from laser (66) is reflected off of adjustably-mounted turning mirrors (68) which direct the laser light to the desired location. Telescope lenses (70) then reduce the beam diameter to approximately 100 μm, and bending mirror (72) directs the light into electrophoresis medium (104) at right angles.

Light emitted from the laser-excited fluorescent label is collected by aspheric collection lens (74) which collimates the light in the direction of the detector. The emitted light then passes around bending mirror (72) and through laser rejection filter (76), thereby reducing the level of scattered laser light entering the detector. Because the excitation laser light passes through the center of aspheric collection lens (74), a certain amount of laser light will be reflected directly back from the lens surface in the direction of the detector, causing unwanted background signal. Bending mirror (72), which is mounted in the center of laser rejection filter (76), acts to deflect this reflected light away from the collection path thus reducing the amount of reflected light entering the detector. The collected emission light then passes through plano-convex lens (78) which focuses the emission light at slit (80) mounted on the entrance to spectrograph (82). (Spectrograph (82) utilizes a 405 g/mm, 450 nm blaze grating with a dispersion of 17 nm/mm.) After passing through spectrograph (82), the light then falls onto CCD (90). The output signal from CCD (90) is transmitted to electronic computer (64) for subsequent data analysis and presentation.

To further increase the emission light signal and decrease background light scatter, a nonconductive mirror coating is applied to inside surface (102) of front gel plate (108). This surface reflects emission light back to the collection lenses rather than allowing it to be lost to the surroundings through the front gel plate. In addition, when the primary laser light strikes this mirrored surface it is reflected back through the gel, thereby exciting additional fluorophores resulting in more emission light. Furthermore, this mirrored surface decreases unwanted background light generated by the fluorescence of the front glass plate itself.

In order to interrogate all of the electrophoresis lanes on a real-time basis, the optical system described above, less turning mirrors (68) and computer (90), is scanned across the width of the electrophoresis chamber.

Another important feature of the present invention is the novel means used to mount the electrophoresis chamber onto the analyzer. Preferably, the electrophoresis chamber is formed by two glass plates separated by two spacers located at the left and right edges of the plates. The glass plates are mounted into a plate holder which acts to support and secure the glass plates along with an upper buffer reservoir in a convenient manner. See Figure 3. The plate holder consists of rectangular frame (200) onto which is attached plurality of twist clamps (204). (Note that only one twist clamp is indicated in Figure 3, as (84), in order to retain the clarity of the drawing.) When twist clamps (84) are in the horizontal orientation, they serve to secure the glass plates in the holder, and, when twist clamps (84) are in a vertical orientation, they allow the glass plates to be conveniently inserted or removed from the plate holder. The rectangular frame includes two locational registration notches (208) to insure the proper positioning of the plate holder in the analyzer. Beam-stop (212) is posi-

tioned so as to protect the user from direct exposure to the excitation laser light. The frame also includes two handles (202) to facilitate transportation of the plate holder assembly. The plate holder provides a means for detachably mounting upper buffer reservoir (216). A protrusion (228) on each side of upper buffer reservoir (216) is positioned such that when the uppermost twist clamps are in the horizontal position, the upper buffer reservoir (216) is forced against the front glass plate, thereby creating a liquid-tight seal between the upper buffer chamber and the front glass plate. Upper buffer reservoir (216) contains electrode (220) and electrical cable (224) for connecting electrode (220) to an electrophoresis power supply. The plate holder is designed to secure glass plates of varying lengths. For applications requiring less separation and/or a shorter analysis time, a shorter length would be used, and for applications requiring more separation and for which longer analysis times can be tolerated, a longer length would be used.

A further important aspect of the present invention is the plate locating mechanism. In order to efficiently collect the fluorescence emission light, the detection region of the electrophoresis chamber must be properly positioned with respect to the collection optics. Specifically, the detection region must be aligned such that the focal point of the collection optics is located within the separation medium, and not in the wall of the electrophoresis chamber. The plate locating mechanism insures that this positioning is reproducibly achieved. The mechanism will be described with reference to Figure 4. When a thin electrophoresis chamber is being used, i. e. less than 0.2 mm, preadjusted locating pins (300) fit through notches (304) in back glass plate (308) and push front glass plate (312) against front tip (324) of locating pins (300). When a thick electrophoresis chamber is being used, i.e. greater than 0.2 mm, step-portion (320) of locating pins (300) is forced against back glass plate (312). Locating pins (300) are preadjusted such that the interior of the electrophoresis chamber is at the focal point of the collection optics. Glass plates (308 and 312) are forced against locating pins (300) by twist clamps (330).

While increasing the electric field across the electrophoresis chamber increases the speed of the electrophoretic separation, it also leads to increased Joule heat generated within the electrophoresis medium, which in turn can lead to destruction of the electrophoresis medium. To remove the heat generated by running "fast" electrophoresis, a temperature control mechanism (Figure 5) has been developed. The temperature control mechanism includes a back heat transfer plate (400) against which back glass plate (404) is mounted to the instrument. Preferably, heat transfer plate (400) is made from coated aluminum. The coating acts as an electrical insulator to inhibit arcing between back glass plate (404) and the rest of the instrument. Within back cooling plate (400) are channels through which a flowable heat transfer medium can be circulated. Front heat transfer plate (408), also containing channels capable of being filled with a flowable heat transfer medium, is contacted with front glass plate (412). Pump (416) circulates the flowable heat transfer medium from reservoir (420) through front and back heat transfer plates (400 and 408). Heat is removed from the circulating flowable heat transfer medium by passing it through heat exchanger (424), thereby cooling the flowable heat transfer medium to ambient temperature. If superambient heating or subambient cooling of the gels is desired for a specific application, the flowable heat transfer medium passes through a heater or cooler (not shown) before flowing through the heat transfer plates. Active temperature control of the gel is effected by means of temperature sensors (430) mounted to the heat transfer plates in combination with computer (434) which regulates the temperature of the plates by controlling the flow rate of the flowable heat transfer medium through the heat transfer plates.

Although the invention has been illustrated by the foregoing description it is not to be construed as being limited to the materials employed therein but rather the invention is directed to the generic area as hereinbefore disclosed. Various modifications and embodiments thereof can be made without departing from the spirit or scope thereof.

Claims

1. A real-time scanning fluorescence electrophoresis apparatus for the electrophoretic analysis of fluorescently-labeled polynucleotide fragments of the type having an electrophoresis chamber containing an electrophoretic separation medium capable of accommodating multiple electrophoresis lanes arranged in a planar array, a fluorescence detector mounted on a translatable stage, a light source for exciting fluorescent molecules, and a computer for collecting data consisting of time, location, fluorescence wavelength and fluorescent intensity information wherein the improvement comprises:

   (a) a spectral-array detector for detecting emission light from said fluorescently-labeled polynucleotide fragments including the simultaneous detection of multiple fluorescent labels,
   (b) a temperature control means to control the temperature of the electrophoretic separation medium during electrophoresis.

2. The apparatus of claim 1 wherein the output of the spectral-array detector is processed so as to effect a virtual filter.

3. The apparatus of claim 2 wherein the wavelengths of said virtual filter are 540, 560, 580, and 610 nm, each 10 nm wide.

4. The apparatus of claim 2 wherein the wavelengths of said virtual filter are 530, 545, 560, 580 nm, each 10 nm wide.

5. The apparatus of claim 1 wherein the spectral-array detector comprises:

(a) a diffraction grating to separate the emission light,
(b) a CCD array to detect the location and intensity of the separated emission light,
(c) a laser excitation light source,
(d) an optical arrangement to direct and condition the excitation and emission light in order to minimize the amount of scattered excitation light reaching the detector.

6. The apparatus of claim 5 wherein the spectral-array detector comprises an optical arrangement comprising:

(a) turning mirrors which direct the laser light to a desired location,
(b) telescopic lenses which focus the laser light to a position within the electophoresis chamber,
(c) a bending mirror that directs the laser light at a right angles to the electrophoresis chamber,
(d) an aspheric collection lens that collimates the fluorescence emission light in the direction of the detector,
(e) a set of laser rejection filters that reduce the level of extraneous laser light entering the detector, and,
(f) a plano-convex lens which focuses the emission light at a desired location.

7. The apparatus of claim 1 wherein the temperature control means comprises thermally controlled front and back heat transfer plates which are in contact with a front and back face of the electrophoresis chamber.

8. The apparatus of claim 7 wherein the front and back heat transfer plates are made from coated aluminum wherein the coating acts to electrically insulate the heat transfer plates from the electrophoresis voltage.

9. The apparatus of claim 7 wherein the temperature control means comprises:

(a) a front heat transfer plate placed in contact with a front face of the electrophoresis chamber, wherein flow channels are formed within the front heat transfer plate including inlet and outlet ports,
(b) a back heat transfer plate placed in contact with a back face of the electrophoresis chamber, wherein flow channels are formed within the front heat transfer plate including inlet and outlet ports,
(c) a flowable heat transfer medium which is circulated through the flow channels in the front and back heat transfer plates,
(d) a pump to circulate the flowable heat transfer medium,
(e) a heat exchanger in which the flowable heat transfer medium can exchange heat with the ambient atmosphere,
(f) a computer for controlling the temperature of the heat transfer plates by controlling the flow of the circulating heat transfer medium,
(g) a temperature sensor in contact with the front and back heat transfer plates and electrically connected to the computer to relay temperature information to the computer.

10. The apparatus of claim 9 wherein said heat exchanger is replaced by a cooler wherein the cooler cools the flowable heat transfer medium below the temperature of the ambient atmosphere.

11. The apparatus of claim 9 wherein said heat exchanger is replaced by a heater wherein the heater heats the flowable heat transfer medium above the temperature of the ambient atmosphere.

12. The apparatus of claim 1 wherein the electrophoresis chamber comprises:

(a) front and a back glass plates, where the back plate is defined as the plate through which the excitation laser light enters the electrophoresis chamber,
(b) two spacers which serve to maintain a uniform separation between the glass plates, spaced so as to provide a chamber thickness of from 0.1 to 1.0 mm,
(c) a plate holder which can accommodate glass plates of varying lengths and which acts to support and secure said electrophoresis medium and wherein said plates are held firmly in place within the plate holder by clamps which keep the edges of the plates sealed to prevent separation medium from leaking.

13. The apparatus of claim 12 having a plate locating mechanism which optimally positions the detection region of the electrophoresis chamber with respect to the detection optics.

14. The apparatus of claim 12 having a mirror coating applied to the inside-facing surface of the front plate so that the excitation laser light, after passing through the back plate and the electrophoresis

chamber, strikes the mirror surface and is reflected back through the electrophoresis chamber, thereby exciting additional fluorophores whose light is then collected, resulting in an increased emitted light signal.

Patentansprüche

1. Eine Vorrichtung zur elektrophoretischen Echtzeitanalyse von fluoreszenzmarkierten Polynucleotidfragmenten durch fluoreszierende Abtastelektrophorese mit einer Elektrophoresekammer, die ein elektrophoretisches Separationsmedium enthält, das mehrere, planar angeordnete Elektrophoresebahnen aufnehmen kann, einem Fluoreszenzdetektor, der auf ein umsetzbares Gestell montiert ist, einer Lichtquelle zur Erregung von Fluoreszenzmolekülen sowie einem Rechner zum Sammeln von Daten über Zeit, Ort, Fluoreszenzwellenlänge und Fluoreszenzintensität, worin die Verbesserung folgendes aufweist:

   (a) einen Spektralanordnungsdetektor zur Erfassung von Emissionslicht aus den fluoreszenzmarkierten Polynucleotidfragmenten, einschließlich simultaner Erfassung mehrfacher Fluoreszenzmarkierungen,

   (b) eine Temperatursteuerung zur Steuerung der Temperatur des elektrophoretischen Separationsmediums während der Elektrophorese.

2. Die Vorrichtung nach Anspruch 1, worin die Ausgabe des Spektralanordnungsdetektor so bearbeitet wird, daß sie einen virtuellen Filter bewirkt.

3. Die Vorrichtung nach Anspruch 2, worin die Wellenlängen des virtuellen Filters 540, 560, 580 und 610 nm sind, jeweils 10 nm breit.

4. Die Vorrichtung nach Anspruch 2, worin die Wellenlängen des virtuellen Filters 530, 545, 560 und 580 nm sind, jeweils 10 nm breit.

5. Die Vorrichtung nach Anspruch 1, worin der Spektralanordnungsdetektor folgendes aufweist:

   (a) ein Beugungsgitter zur Separation des Emissionslichts,

   (b) eine CCD-Anordnung zur Erfassung von Ort und Intensität des separierten Emissionslichts,

   (c) eine Laser-Erregerlichtquelle,

   (d) einen optischen Aufbau, um das Erreger- und Emissionslicht so zu lenken und zu konditionieren, daß die Menge an zerstreutem Erregerlicht, das den Detektor erreicht, minimiert wird.

6. Die Vorrichtung nach Anspruch 5, worin der Spektralanordnungsdetektor einen optischen Aufbau aufweist, der folgendes aufweist:

   (a) Drehspiegel, die das Laserlicht an einen gewünschten Ort lenken,

   (b) Teleskoplinsen, die das Laserlicht auf eine Position innerhalb der Elektrophoresekammer fokussieren,

   (c) einen Krümmungsspiegel, der das Laserlicht in einem rechten Winkel zur Elektrophoresekammer lenkt,

   (d) eine aspherische Sammellinse, die das Fluoreszenzemissionslicht in Richtung des Detektors kollimiert,

   (e) einen Satz laserabweisender Filter, welche die Menge externen Laserlichts, das in den Detektor eintritt, verringern, sowie

   (f) eine plankonvexe Linse, die das Emissionslicht an einem gewünschten Ort fokussiert.

7. Die Vorrichtung nach Anspruch 1, worin die Temperatursteuerung thermisch gesteuerte stimseitige und rückseitige Wärmeübertragungsplatten aufweist, die eine stirnseitige und eine rückseitige Fläche der Elektrophoresekammer berühren.

8. Die Vorrichtung nach Anspruch 7, worin die stirnseitige und die rückseitige Wärmeübertragungsplatte aus beschichtetem Aluminium bestehen, worin die Beschichtung so wirkt, daß sie die Wärmeübertragungsplatten von der Elektrophoresespannung elektrisch isoliert.

9. Die Vorrichtung nach Anspruch 7, worin die Temperatursteuerung folgendes aufweist:

   (a) eine stirnseitige Wärmeübertragungsplatte, die eine stirnseitige Fläche der Elektrophoresekammer berührt, worin Durchflußkanäle mit Einund Auslaßöffnungen in der stirnseitigen Wärmeübertragungsplatte ausgebildet sind,

   (b) eine rückseitige Wärmeübertragungsplatte, die eine rückseitige Fläche der Elektrophoresekammer berührt, worin Durchflußkanäle mit Ein- und Auslaßöffnungen in der stirnseitigen Wärmeübertragungsplatte ausgebildet sind,

(c) ein fließfähiges Wärmeübertragungsmedium, das durch die Durchflußkanäle in der stirnseitigen und der rückseitigen Wärmeübertragungsplatte zirkuliert,

(d) eine Pumpe zur Zirkulation des fließfähigen Wärmeübertragungsmediums,

(e) einen Wärmetauscher, in dem das fließfähige Wärmeübertragungsmedium Wärme mit der umgebenden Atmosphäre austauschen kann,

(f) einen Rechner zur Steuerung der Temperatur der Wärmeübertragungsplatten durch Steuerung des Durchflusses des zirkulierenden Wärmeübertragungsmediums,

(g) einen Temperatursensor, der die stirnseitige und die rückseitige Wärmeübertragungsplatte berührt und elektrisch mit dem Rechner verbunden ist, um Temperaturdaten an den Rechner zu übertragen.

10. Die Vorrichtung nach Anspruch 9, worin der Wärmetauscher durch eine Kühlvorrichtung ersetzt wird, worin die Kühlvorrichtung das fließfähige Wärmeübertragungsmedium unter die Temperatur der umgebenden Atmosphäre abkühlt.

11. Die Vorrichtung nach Anspruch 9, worin der Wärmetauscher durch eine Heizvorrichtung ersetzt wird, worin die Heizvorrichtung das fließfähige Wärmeübertragungsmedium über die Temperatur der umgebenden Atmosphäre aufheizt.

12. Die Vorrichtung nach Anspruch 1, worin die Elektrophoresekammer folgendes aufweist:

(a) eine stirnseitige und eine rückseitige Glasplatte, wobei die rückseitige Platte als die Platte definiert ist, durch die das Erreger-Laserlicht in die Elektrophoresekammer eintritt,

(b) zwei Abstandshalter, die dazu dienen, einen gleichmäßigen Abstand zwischen den Glasplatten einzuhalten und die soweit voneinander entfernt sind, daß eine Kammerdicke von 0,1 bis 1,0 mm entsteht,

(c) einen Plattenhalter, der Glasplatten von unterschiedlicher Länge aufnehmen kann und so wirkt, daß er das Elektrophoresemedium stützt und festhält, und worin die Glasplatten durch Zwingen, welche die Ränder der Platten abdichten, um ein Lecken des Separationsmediums zu verhindern, sicher an ihrem Platz im Plattenhalter festgehalten werden.

13. Die Vorrichtung nach Anspruch 12 mit einem Plattenjustierungsmechanismus, der den Detektionsbereich der Elektrophoresekammer im Verhältnis zur Detektionsoptik optimal positioniert.

14. Die Vorrichtung nach Anspruch 12, worin eine Spiegelbeschichtung auf die nach innen gekehrte Fläche der stirnseitigen Platte aufgetragen ist, so daß das Erreger-Laserlicht nach dem Durchtritt durch die rückseitige Platte und die Elektrophoresekammer auf die Spiegelfläche trifft und zurück durch die Elektrophoresekammer reflektiert wird, wodurch es zusätzliche Fluorophoren erregt, deren Licht dann gesammelt wird, was zu einem stärkeren emittierten Lichtsignal führt.

Revendications

1. Appareil d'électrophorèse à fluorescence à balayage en temps réel, pour l'analyse électrophorétique de fragments polynucléotidiques marqués par fluorescence, du type possèdant une chambre d'électrophorèse contenant un milieu de séparation électrophorétique pouvant accueillir plusieurs rangées d'électrophorèse disposées selon une configuration plane, un détecteur de fluorescence monté sur une plate-forme pouvant être déplacée en translation, une source de lumière pour exciter des molécules fluorescentes et un ordinateur pour collecter des données constituées par des informations de temps, de position, de longueur d'onde de fluorescence, et d'intensité de fluorescence, dans lequel le perfectionnement comprend :

(a) un détecteur de réseau spectral pour la détection de la lumière d'émission en provenance desdits fragments polynucléotidiques marqués par fluorescence y compris la détection simultanée de plusieurs marqueurs fluorescents,
(b) un moyen de contrôle de la température pour contrôler la température du milieu de séparation électrophorétique au cours de l'électrophorèse.

2. Appareil selon la revendication 1, dans lequel la sortie du détecteur de réseau spectral est traitée de manière à constituer un filtre virtuel.

3. Appareil selon la revendication 2, dans lequel les longueurs d'onde dudit filtre virtuel sont 540, 560, 580 et 610 nm, chacune ayant une largeur de 10 nm.

4. Appareil selon la revendication 2, dans lequel les longueurs d'onde dudit filtre virtuel sont 530, 545, 560, et 580 nm, chacune ayant une largeur de 10 nm.

5. Appareil selon la revendication 1, dans lequel le détecteur de réseau spectral comprend :

(a) un réseau de diffraction pour séparer la lumière d'émission,
(b) une matrice de CCD (circuit à couplage de charges) pour détecter la position et l'intensité de la lumière d'émission séparée,
(c) une source laser de lumière d'excitation,
(d) un dispositif optique pour diriger et conditionner la lumière d'excitation et d'émission afin de minimiser la quantité de lumière d'excitation diffusée atteignant le détecteur.

6. Appareil selon la revendication 5, dans lequel le détecteur de réseau spectral comprend un dispositif optique comportant :

(a) des miroirs rotatifs qui dirigent la lumière laser vers une position désirée,
(b) des lentilles télescopiques qui focalisent la lumière laser vers une position située à l'intérieur de la chambre d'électrophorèse,
(c) un miroir de déflexion qui dirige la lumière laser perpendiculairement à la chambre d'électrophorèse,
(d) une lentille collectrice asphérique qui collimate la lumière d'émission par fluorescence dans la direction du détecteur,
(e) un groupe de filtres d'atténuation de laser réduisant le niveau de lumière laser parasite qui entre dans le détecteur, et
(f) une lentille plan-convexe qui focalise la lumière d'émission au niveau d'une position désirée.

7. Appareil selon la revendication 1, dans lequel le moyen de contrôle de la température comprend des plaques de transfert thermique avant et arrière qui sont en contact avec une face avant et une face arrière de la chambre d'électrophorèse.

8. Appareil selon la revendication 7, dans lequel les plaques de transfert thermique avant et arrière sont faites d'aluminium pourvu d'un revêtement, le revêtement ayant pour but d'isoler électriquement les plaques de transfert thermique vis-à-vis de la tension d'électrophorèse.

9. Appareil selon la revendication 7, dans lequel le moyen de contrôle de la température comprend :

(a) une plaque de transfert thermique avant mise en contact avec une face avant de la chambre d'électrophorèse, des canaux d'écoulement étant formés à l'intérieur de la plaque de transfert thermique avant, y compris des orifices d'entrée et de sortie,
(b) une plaque de transfert thermique arrière mise en contact avec une face arrière de la chambre d'électrophorèse, des canaux d'écoulement étant formés à l'intérieur de la plaque de transfert thermique arrière, y compris des orifices d'entrée et de sortie,
(c) un milieu de transfert thermique fluide qui circule à travers les canaux d'écoulement dans les plaques de transfert avant et arrière,
(d) une pompe pour faire circuler le milieu de transfert thermique fluide,
(e) un échangeur de chaleur dans lequel le milieu de transfert thermique fluide peut échanger de la chaleur avec l'atmosphère ambiante,
(f) un ordinateur pour contrôler la température des plaques de transfert thermique par un contrôle de l'écoulement du milieu de transfert thermique en circulation,
(g) un capteur de température en contact avec les plaques de transfert thermique avant et arrière et relié électriquement à l'ordinateur afin de transmettre des données de température à l'ordinateur.

10. Appareil selon la revendication 9, dans lequel l'échangeur de chaleur est remplacé par un dispositif de refroidissement, l'appareil de refroidissement portant le milieu de transfert thermique fluide à une température inférieure à celle de l'atmosphère ambiante.

11. Appareil selon la revendication 9, dans lequel ledit échangeur de chaleur est remplacé par un dispositif de chauffage, le dispositif de chauffage portant le milieu de transfert thermique fluide à une température supérieure à celle de l'atmosphère ambiante.

12. Appareil selon la revendication 1, dans lequel la chambre d'électrophorèse comprend :

(a) des plaques de verre avant et arrière, la plaque arrière étant définie comme étant la plaque à travers laquelle la lumière laser d'excitation entre dans la chambre d'électrophorèse,
(b) deux pièces d'écartement qui servent à maintenir une séparation uniforme entre les plaques de verre, espacées de manière à procurer une épaisseur de chambre de 0,1 à 1,0 mm,
(c) un support de plaques qui peut recevoir des plaques de verre de longueurs différentes et qui sert à supporter et à retenir ledit milieu d'électrophorèse et dans lequel lesdites plaques sont fermement maintenues en place à l'intérieur du support de plaques par des fixations qui maintiennent les bords des plaques étanches afin d'empêcher des fuites du milieu de séparation.

13. Appareil selon la revendication 12 possédant un mécanisme de positionnement de plaque qui positionne de manière optimale la zone de détection de la chambre d'électrophorèse par rapport à l'optique de détection.

14. Appareil selon la revendication 12 possédant un revêtement de miroir appliqué sur la surface de la plaque avant qui est tournée vers l'intérieur, de sorte que la lumière laser d'excitation, après avoir traversé la plaque arrière et la chambre d'électrophorèse, rencontre la surface du miroir et renvoyée par réflexion à travers la chambre d'électrophorèse pour ainsi exciter des fluorophores supplémentaires dont la lumière est récupérée, avec pour résultat un signal lumineux émis accru.

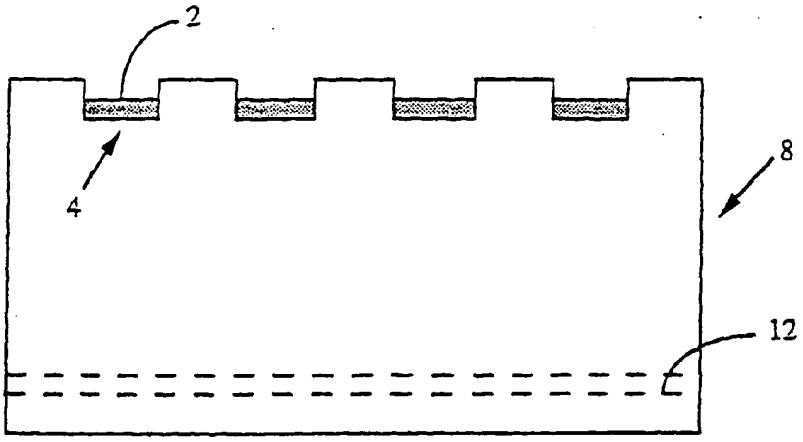

Fig. 1

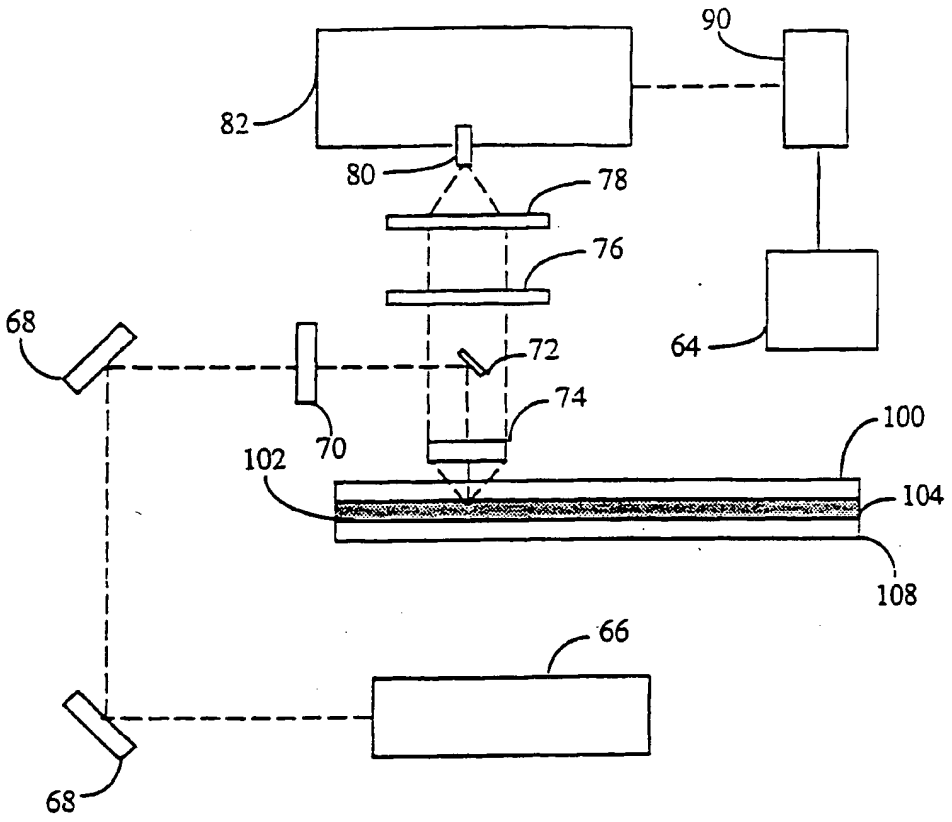

Fig. 2

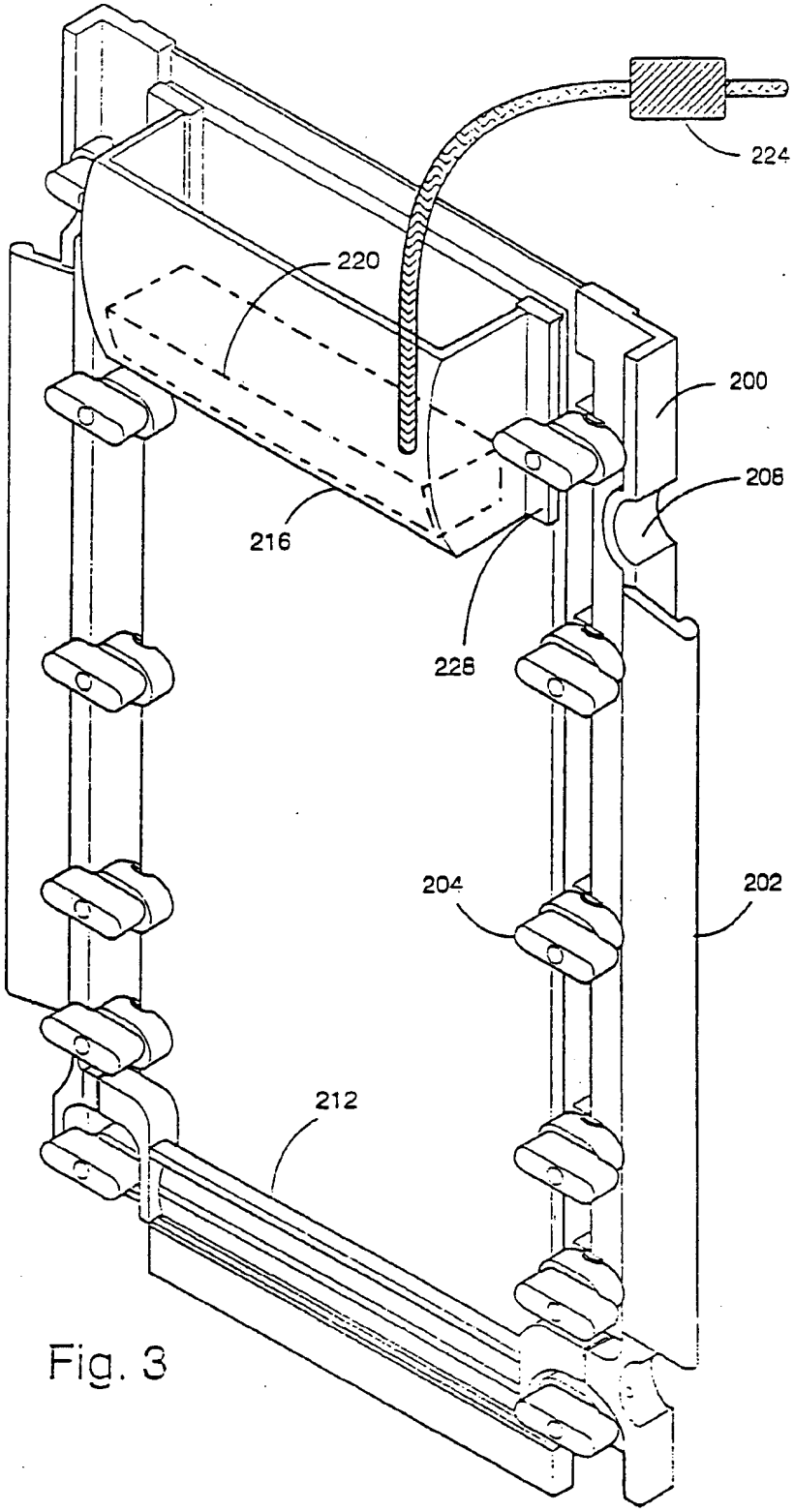

Fig. 3

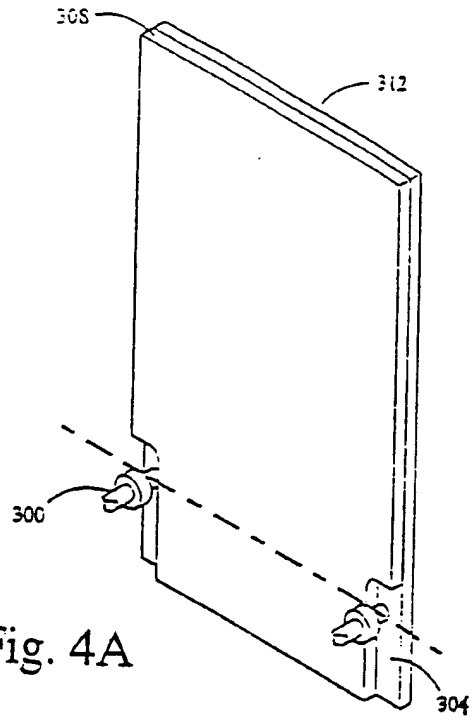

Fig. 4A

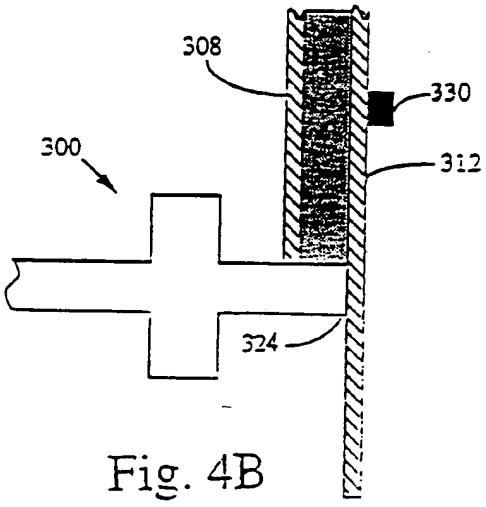

Fig. 4B

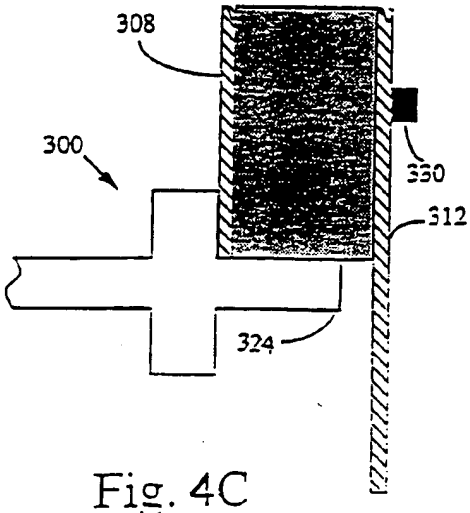

Fig. 4C

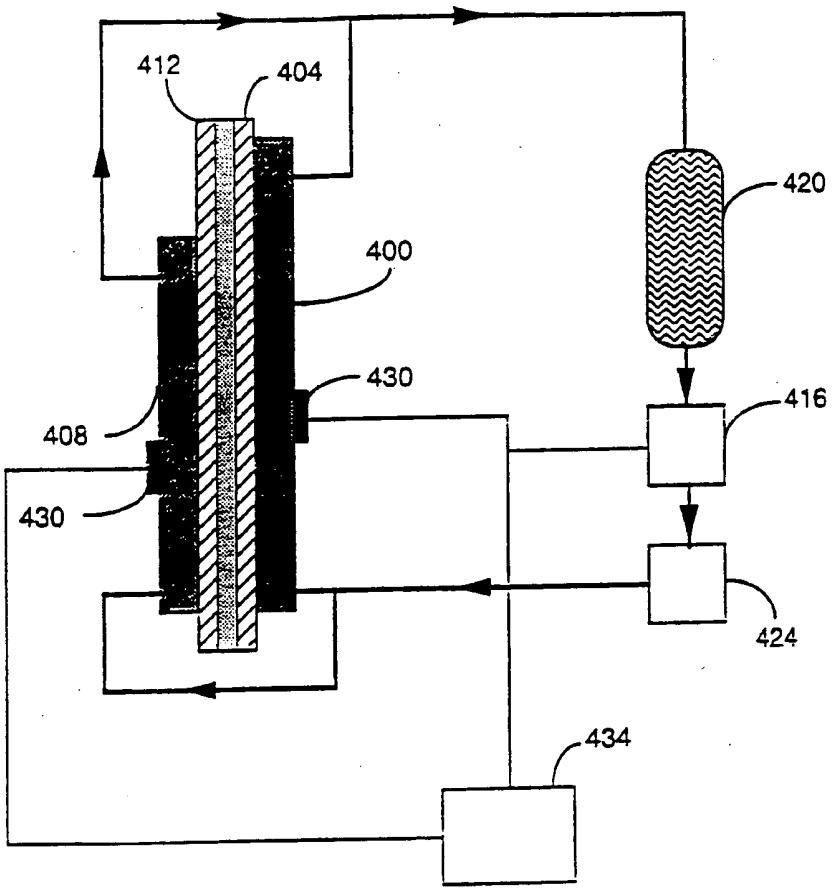

Fig. 5